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Sample Handling and Analysis Method for Chemical Warfare Agents in Soils Contaminated with Chemical and/or Biological Warfare Agents

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Defence R&D Canada – Suffield

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Abstract

A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated using three soil samples spiked at the 80 μg/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure. This sample handling and analysis method for soil (or other) samples will form a cornerstone of the chemical/biological warfare agent identification strategy being developed at DRDC Suffield to meet National commitments.

Résumé

Une méthode pour manipuler et analyser les échantillons a été mise au point et vise à identifier les agents chimiques de guerre (comme composés intacts ou comme les produits de leur hydrolyse) dans des échantillons suspects de contenir des agents de contamination biologique ou chimique de guerre. La méthode, basée sur une extraction aqueuse, la stérilisation en autoclave d'un extrait aqueux et une analyse CPL- IPE- SM (chromatographie en phase liquide – ionisation par pulvérisation d'électrons -spectrométrie de masse), a été évaluée en utilisant trois échantillons de sols semés à un niveau de 80 μg/g avec du phosphate d'éthyle, de l'acide isopropyle méthylphosphonique ou du thiodiglycol. Ces trois composés ont été facilement extraits et identifiés, après l'analyse CPL-IPE-SM, avec le phosphate d'éthyle subissant une hydrolyse durant la procédure de stérilisation en autoclave. Cette méthode de manipulation et d'analyse d'échantillons pour les sols (ou autres) sera une étape primordiale dans la stratégie d'identification d'agent de guerre chimique et biologique qui est mise au point à RDDC Suffield et vise à répondre aux engagements souscrits par le pays.

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Executive summary

Introduction: The Canadian Forces (CF) may be called on to perform peacekeeping or battlefield operations in regions of the world where there is a significant threat of chemical/biological (CB) warfare agent use. To operate effectively in these theatres the CF must be able to identify the CB agent used. Mass spectrometry (MS), is a powerful analytical technique for the identification of both known and unknown compounds and DRDC Suffield is currently investigating this instrumental technique in fulfillment of CF detection and identification requirements.

Results: A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated using three soil samples spiked at the 80 μg/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure.

Significance: A sample handling and analysis method for soil (or other) samples was developed as part of a chemical/biological warfare agent identification strategy for the G8 Summit held in Kananaskis (2002). This sample handling and analysis method for soil (or other) samples will form a cornerstone of the CB warfare agent identification strategy being developed at DRDC Suffield to meet CF and National commitments and will be incorporated into the Standard Operating Procedures of the DRDC Suffield Chemical Biological Forensic Reference Laboratory.

Future Plans: The reported method will be a valuable addition to the present methods for the identification of chemical warfare agents and their hydrolysis products in samples collected by the Canadian Forces, RCMP or in support of Chemical Weapons Convention challenge inspections. The application of tandem mass spectrometry to sample containing CB contamination is anticipated upon installation of a new instrument at DRDC Suffield.

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Sommaire

Introduction: Les Forces canadiennes (FC) peuvent être appelées à entreprendre des opérations de maintien de la paix ou de champ de bataille dans des régions du monde où existe une menace importante d'utilisation d'agents chimiques et biologiques de guerre (CB). Pour être capable d'opérer efficacement dans ces théâtres, les FC doivent être capables d'identifier les agents CB utilisés. La spectrométrie de masse (SM) est une technique analytique puissante pour l'identification des composés connus et inconnus et RDDC Suffield examine actuellement cette technique qui est instrumentale pour satisfaire aux besoins de détection et d'identification des FC.

Résultats : Une méthode pour manipuler et analyser les échantillons a été mise au point et vise à identifier les agents chimiques de guerre (comme composés intacts ou les produits de leur hydrolyse) dans des échantillons suspects de contenir des agents de contamination de guerre biologique ou chimique. La méthode, basée sur une extraction aqueuse, la stérilisation en autoclave d'un extrait aqueux et une analyse CPL-IPE-SM, a été évaluée en utilisant trois échantillons de sols semés à un niveau de 80 µg/g avec du phosphate d'éthyle, de l'acide isopropyle méthylphosphonique ou du thiodiglycol. Ces trois composés ont été facilement extraits et identifiés selon CPL- IPE- SM avec le phosphate d'éthyle subissant une hydrolyse durant la procédure de stérilisation en autoclave. Cette méthode de manipulation et d'analyse d'échantillons pour les sols (ou autres) sera une étape primordiale dans la stratégie d'identification d'agents de guerre chimiques et biologiques qui est mise au point à RDDC Suffield et vise à répondre aux engagements souscrits par le pays.

Portée des résultats : Une méthode de manipulation et d'analyse d'échantillons pour les sols (ou autres) a été mise au point comme faisant partie d'une stratégie d'identification d'agents de guerre chimiques et biologiques proposée au Sommet du G8 qui s'est tenu à Kananaski, en 2002. Cette méthode de manipulation et d'analyse d'échantillons pour les sols (et autres) sera une étape importante dans la stratégie d'identification des agents de guerre CB. Cette stratégie est mise au point à RDDC Suffield ; elle vise à répondre aux engagements souscrits par le pays et sera incorporée dans le mode opératoire normalisé du Laboratoire de référence chimique et biologique médico-légal du RDDC Suffield.

Planification future : Cette méthode apporte un complément important aux méthodes actuelles en ce qui concerne l'identification des agents chimiques de guerre et des produits de leur hydrolyse en échantillons recueillis par les Forces canadiennes ou la GRC ainsi qu'aux inspections par mise en demeure de la Convention sur les armes chimiques. On prévoit d'appliquer la spectrométrie de masse en tandem aux échantillons contaminés par des agents CB dès l'installation d'un nouvel instrument à RDDC Suffield.

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Table of contents

List of figures

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Introduction

More than 140 States Parties have ratified the Chemical Weapons Convention (CWC) and agreed not to develop, produce, stockpile, transfer or use chemical weapons and agreed to destroy their own chemical weapons and production facilities. The CWC has reduced the likelihood of chemical weapons use by States Parties, but there remains a serious concern that other parties may make use of these weapons against civilian or military targets. Methods need to be developed to ensure that suspect samples collected under these scenarios can be analysed for the presence of chemical warfare agents in a timely manner. These analytical demands are being actively addressed by DRDC Suffield through the development and application of new mass spectrometric (MS) methods for the detection and identification of chemical warfare agents in a variety of samples.

Gas chromatography (GC) has been used extensively for the separation and identification of chemical warfare agents, with GC-MS being used frequently for the characterization of these compounds [1,2]. GC-MS, while suitable for the direct analysis of organophosphorus chemical warfare agent in organic extracts, is usually not preferred for the direct analysis of aqueous samples or extracts since these samples normally require additional sample handling steps and derivatization prior to analysis. LC-ESI-MS is being used increasingly, as electrospray mass spectrometric data may be used to directly identify chemical warfare agents, degradation products and related compounds in collected aqueous samples or extracts.

Researchers have developed atmospheric pressure ionization (e.g., electrospray (ESI), ionspray and atmospheric pressure chemical ionization) methods for the characterization of polar pesticides [3], organophosphate esters [4], and chemical warfare agents and/or their degradation products [5-25]. These ionization modes have been interfaced to liquid chromatography (LC) and capillary electrophoresis (CE), with LC-MS [9-12, 14, 15, 18-24] and CE-MS [5, 13] methods being reported for the identification of lower volatility chemical warfare agent hydrolysis products. Recently, DRDC Suffield published a number of LC-ESI-MS papers on the simultaneous identification of organophosphorus chemical warfare agents and their hydrolysis products in aqueous (or snow) samples [14-17, 20, 23-25] and aqueous extracts of contaminated soil samples [21, 22]. The ESI-MS data acquired during these and other analyses have been compiled into an ESI-MS database [26] that may be used for identification purposes.

A particularly challenging problem from a sample handling and analysis viewpoint has been the development of a safe procedure for the analysis of chemical warfare agents in samples where biological warfare agent contamination is also suspected. Analysis for chemical warfare agent contamination can only take place after the sample has been deemed free of biological warfare agent contamination. Unfortunately, the time required for culturing experiments to prove the absence of biological activity may take up to two weeks. A more rapid sample handling and analysis method on these types of unknown samples is required.

DRDC Suffield's recent experiences with aqueous samples and extracts, and their analyses by LC-ESI-MS [11-12, 14-17, 20-26] enabled the development of a new sample handling and analysis method for samples where the chemical/biological contamination was unknown. Soil, a typical environmental sample, was selected to evaluate this analytical approach which

utilizes aqueous extraction, autoclaving to free the sample of biological activity and LC-ESI-MS for compound confirmation. Ottawa sand samples were spiked at the 80 μg/g level with triethyl phosphate (a chemical warfare agent simulant that is resistant to hydrolysis), isopropyl methylphosphonic acid (the initial hydrolysis product of sarin) or thidiglycol (the hydrolysis product of mustard). This spiking level was below typical battlefield contamination levels, estimated to be in the 100 to 1000 μg/g range, based on a contamination density of 1 to 10 g/m^2 (soil density about 1 g/cm^3 and a 1 cm sampling depth) and considered typical of soil contamination levels that might be expected hours to days after an attack. Soil samples were extracted with water using ultrasonic vibration and a portion of the aqueous extract was removed and autoclaved to eliminate biological activity. This aqueous extract was centrifuged and analysed by LC-ESI-MS to confirm compound identity.

Experimental

Sample handling

Three Ottawa sand samples (3.0 g) were each weighed into 15 x 125 mm screw-capped Teflon-lined glass culture tubes. The sand samples were then spiked with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol (25 µL aliquot of a 10 mg/mL solution in water) and allowed to stand at room temperature for one hour to simulate collected contaminated soil samples.

The spiked samples were ultrasonically extracted with water (10 mL) in the glass culture tubes for 10 minutes and then centrifuged in the same tubes at 2000 rpm for 10 minutes to settle out most of the sand. An aliquot of the aqueous layer (6 to 7 mL) was removed and transferred into a screw-capped Teflon-lined 20 mL glass scintillation vial. The lid was left finger-tight and the vial was autoclaved for two hours at 121°C at 15 psi (liquid cycle). The sterilized aqueous extract was allowed to cool and an aliquot (1 mL) was removed, transferred into a 1.5 mL plastic microcentrifuge tube, and centrifuged for 10 min at 10,000 rpm. A portion of the resulting extract was removed and stored in a 1.8 mL screw-capped Teflon-lined glass sample vial prior to LC-ESI-MS analysis.

An aliquot (1 mL) from the initial aqueous soil extracts was taken prior to autoclaving for comparative purposes. It was transferred into a 1.5 mL plastic microcentrifuge tube, and centrifuged for 10 minutes at 10,000 rpm. A portion of the resulting extract was removed and stored in a 1.8 mL screw-capped Teflon-lined glass sample vial prior to LC-ESI-MS analysis.

Biocontainment level 3 (BL-3) sample handling equipment list

1. analytical balance
2. Teflon-lined glass culture tubes
3. disposable scoopulas
4. water (extraction solvent)
5. 5 and 10 mL disposable pipettes and bulb
6. ultrasonic bath (or vortex)
7. Teflon-lined glass scintillation vials
8. lab marker
9. chemical waste jar
10. methanol/KOH decontamination solution

LC-ESI-MS analysis

LC separations were performed with a MicroTech 150 mm x 0.32 mm i.d fused-silica capillary column packed with Zorbax C_{18} SB (5 μm particle size). The sample was introduced onto the column with a Rheodyne 8125 manual injector equipped with a 5 μL sample loop. The following solvent compositions were prepared for the mobile phase: Solvent A (0.1% trifluoroacetic acid (TFA) in water) and Solvent B (0.1% TFA in acetonitrile/water, 95:5). Chromatographic separations were performed with an Applied Biosystems model 140B dual syringe pump using a 5% to 75%B gradient over 30 minutes. In order to minimize dead volume effects and ensure reproducible mixing, the mobile phase was delivered at 150 μL/min and split prior to the injector such that the flow through the column was 10 μL/min.

LC-ESI-MS data were acquired using a Micromass LCT time-of-flight mass spectrometer equipped with the Z-spray electrospray interface. The electrospray capillary was operated at 3.2 kV with a sampling cone voltage of 20 volts. Nitrogen desolvation gas was introduced into the interface (80 °C) at a flow rate of 480 L/h. Nitrogen nebulizer gas was introduced at a flow rate of 66 L/h. ESI-MS data were acquired from 70 to 700 Da (1 sec) in the continuum mode with a resolution of 5000 (50% valley definition).

Results and discussion

Samples that may be contaminated with a combination of chemical and/or biological warfare agents pose a special problem to chemical and biological specialists tasked with determining the presence of chemical or biological warfare agents. Such a sample would initially be received into biocontainment level 3 (BL-3) at DRDC Suffield where biological identification may be safely carried out. Under normal circumstances, a sample extract requiring removal from BL-3 for use in a BL-2 or chemical laboratory would be sterilized by 0.22 μm filtration. A sterility check of the filtered extract would be conducted in BL-3, a process that may take up to two weeks. During this time chemical detection within BL-3 would be limited to devices such as the Chemical Agent Monitor. A more rapid sample handling and analysis method that could determine the presence (or absence) of chemical warfare agent contamination would be valuable to the military or during crime scene investigations. In addition, rapid determination of the absence of chemical warfare agent would benefit those working in BL-3 as the level of precautions required could be reduced.

The most rapid and effective means of sterilizing a sample contaminated with biological warfare agents that allows removal of the sample from BL-3, without a sterility check, involves autoclaving the sample for 2 hours. Any sample undergoing this process is necessarily exposed to water vapour at a high temperature, making the likelihood of chemical warfare agent hydrolysis high. An analytical method for chemical warfare agent identification must therefore be able to identify the principal hydrolysis products of the common chemical warfare agents. LC-ESI-MS may be used for this purpose and has the added benefit of being able to also detect and identify intact organophosphorus chemical warfare agents and many related compounds in aqueous sample extracts [26].

The developed sample handling method involves aqueous extraction of the sample (e.g., soil) in BL-3, followed by sterilization of the aqueous extract in the autoclave between BL-3 and the rest of the building. The sterilized container and aqueous contents can then be safely manipulated in the chemical analysis laboratory and analysed for the presence or absence of chemical warfare agents, their hydrolysis products or related compounds.

Three compounds were selected for evaluation of the proposed sample handling method, based in part on DRDC Suffield experiences with aqueous extraction and analysis of contaminated soil samples [21, 22]. Triethyl phosphate, an organophorous compound that has been used as a nerve agent simulant, was selected to investigate the extent of hydrolysis during autoclaving, as this compound is much more resistant to hydrolysis than the common organophosphorus chemical warfare agents. Isopropyl methylphosphonic acid was selected as a typical organophosphorus chemical warfare agent hydrolysis product and to investigate the likelihood of hydrolysis to methylphosphonic acid. Finally thiodiglycol was selected since this product would be expected following mustard hydrolysis. It should be noted that mustard cannot be readily detected by LC-ESI-MS, but hydrolyses readily in the presence of water to thiodiglycol, a compound that may be detected by LC-ESI-MS.

Figure 1 illustrates typical chromatograms obtained during LC-ESI-MS analysis of the three spiked soil samples. All three spiked compounds were readily extracted from the Ottawa sand

(recovery efficiencies were not estimated) at the 80 μg/g level. Triethyl phosphate underwent some hydrolysis (about 10-20%) to diethyl hydrogen phosphate, suggesting that hydrolysis of organophosphorus chemical warfare agents to their initial acids would be significant following autoclaving. Hydrolysis of isopropyl methylphosphonic acid to methylphosphonic acid, the common hydrolysis product for many of the organophospohorus chemical warfare agents, was not observed and no additional products were observed in the aqueous extract containing thiodiglycol. Figure 2 and 3 illustrate typical ESI-MS data obtained for each of the spiked compounds and diethyl hydrogen phosphate with a lower sampling cone voltage.

The chromatograms obtained for the aqueous extracts that did not undergo autoclaving were similar with two exceptions. The concentration of the spiked analyte was noticeably lower in these samples since the aqueous extracts underwent a 20% volume reduction during autoclaving. Peak area measurement comparisons suggested that there was little loss of analyte during the autoclave step. Finally, no hydrolysis of triethyl phosphate was found to occur in the spiked aqueous extracts that did not undergo autoclaving, a finding that was consistent with prior experiences.

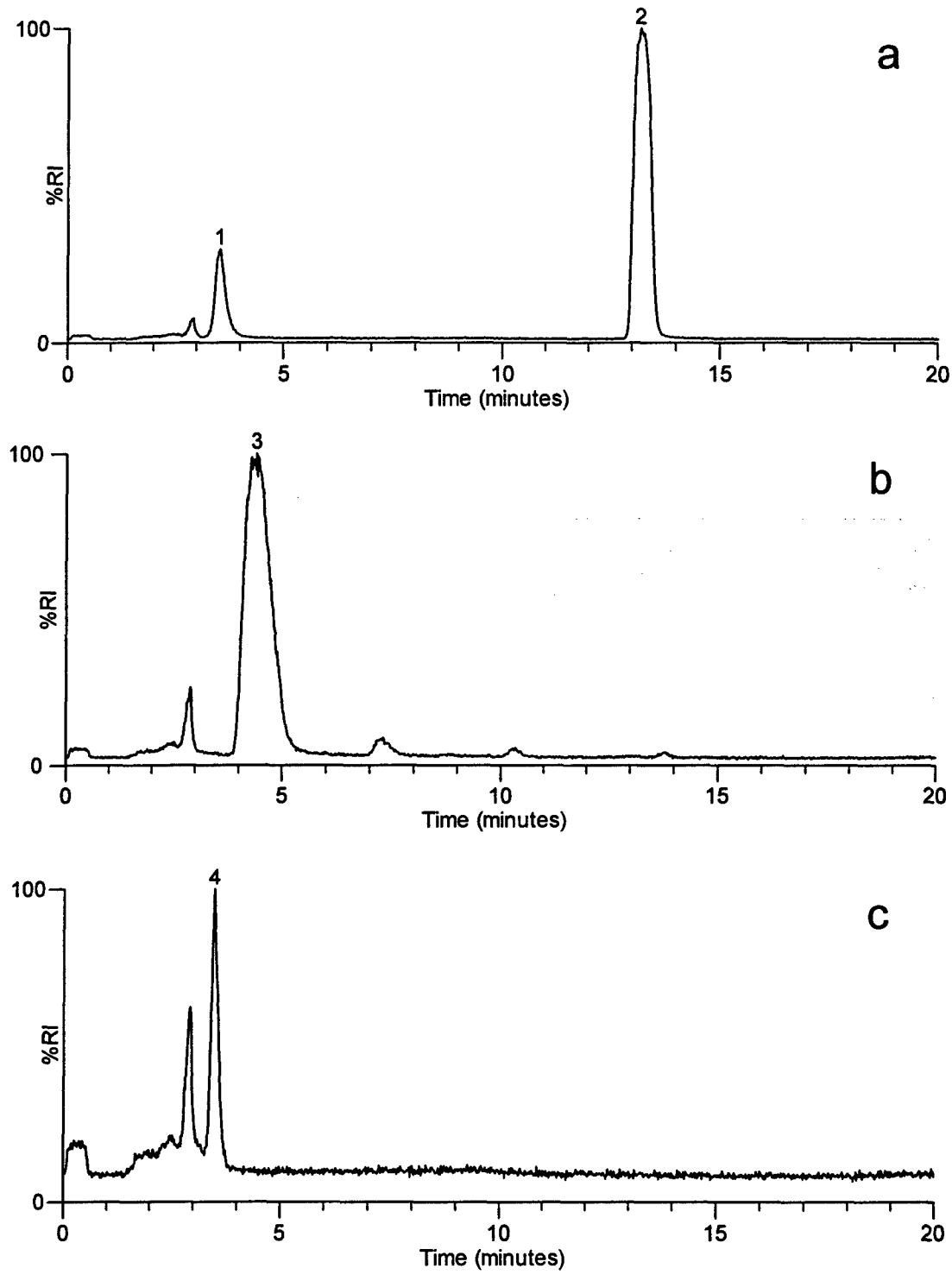

Figure 1. *LC-ESI-MS total-ion-current (90 to 200 Da) chromatograms obtained for the autoclaved aqueous extracts of the Ottawa sand samples spiked at the 80 μg/g level with a) triethyl phosphate (peak # 2), b) isopropyl methylphosphonic acid (peak # 3) and c) thiodiglycol (peak # 4). Diethyl hydrogen phosphate (peak # 1), the initial hydrolysis product of triethyl phosphate, was formed during autoclaving.*

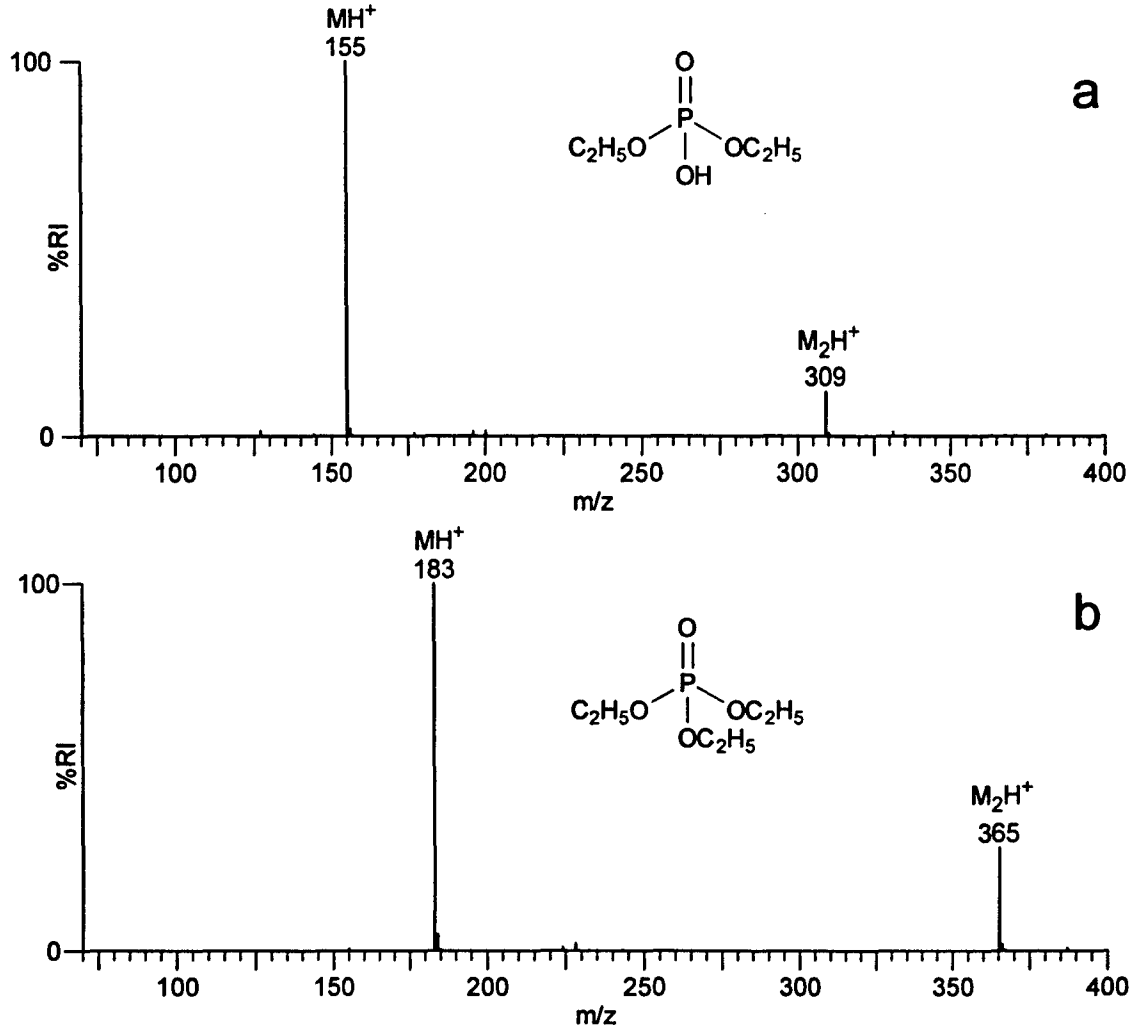

Figure 2. *Typical ESI-MS data (sampling cone: 20V) obtained for a) diethyl hydrogen phosphate and b) triethyl phosphate during LC-ESI-MS analysis of the autoclaved aqueous extracts of the spiked Ottawa sand samples.*

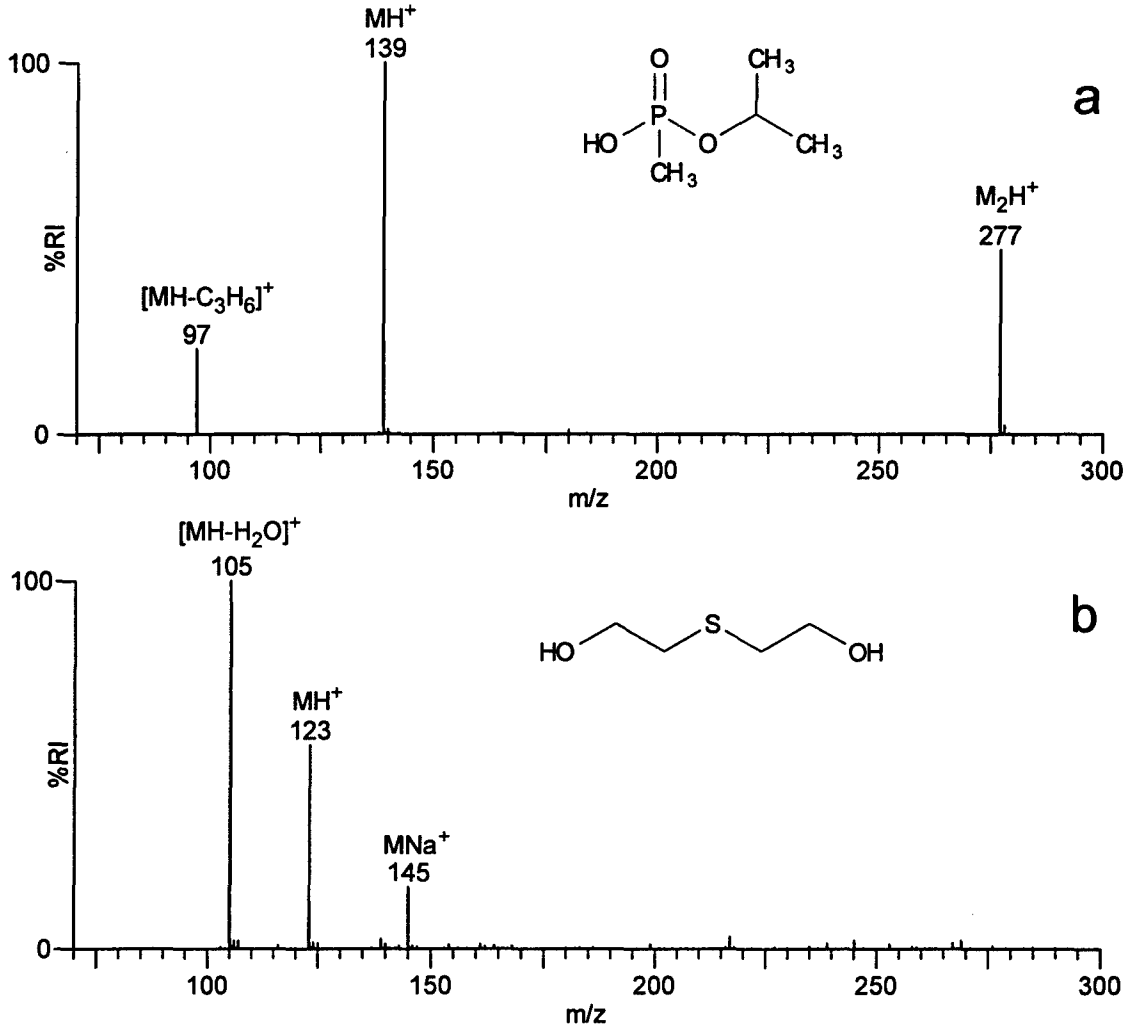

Figure 3. *Typical ESI-MS data (sampling cone: 20V) obtained for a) isopropyl methylphosphonic acid and b) thiodiglycol during LC-ESI-MS analysis of the autoclaved aqueous extracts of the spiked Ottawa sand samples.*

Conclusions

A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated with three soil samples spiked at the 80 μg/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three spiked compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure. The sample handling and analysis method for soil (or other) samples was developed as part of a chemical/biological warfare agent identification strategy for the G8 Summit held in Kananaskis (2002). This method is now being incorporated into the Standard Operating Procedures of the DRDC Suffield Chemical Biological Forensic Reference Laboratory which will begin operation in 2003.

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A sample handling and analysis method was developed for the identification of chemical warfare agents (as intact compounds or as their hydrolysis products) in samples suspected to contain either chemical or biological warfare agent contamination. The method, based on aqueous extraction, autoclaving of the aqueous extract and LC-ESI-MS analysis was evaluated using three soil samples spiked at the 80 μg/g level with triethyl phosphate, isopropyl methylphosphonic acid or thiodiglycol. All three compounds were readily extracted and identified following LC-ESI-MS, with triethyl phosphate undergoing some hydrolysis during the autoclaving procedure. This sample handling and analysis method for soil (or other) samples will form a cornerstone of the chemical/biological warfare agent identification strategy being developed at DRDC Suffield to meet National commitments.

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Chemical warfare agents
Organophosphorus compounds
Sarin
G-agents
Liquid chromatography
Mass spectrometry
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